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SEEDLING BLIGHT AND STACK-BURN OF RICE AND THE HOT-WATER SEED TREATMENT.

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INTRODUCTION.

The rice crop of the southern part of the United States suffers heavy annual losses due to the staining and decay of the grain in the shock and in storage, both before and after milling, and to poor germination of the seed when sown. The former is much more important than the latter. Occasionally fields have to be reseeded, and others have poor stands. The milled product, on the other hand, is considered of inferior quality if only a low percentage of the grain is flecked or decayed. Van der Wolk,[1] in discussing the trouble known to the European rice trade as "yellow grains," says that it is "one of the most fearful calamities in the trade rice of some countries." "Yellow grains" is similar to the stains which develop in our southern rice. It is caused by a different fungus, which accounts for the difference in color. The disease appears in our American rices as flecks or totally decayed grains which are yellowish brown to dark brown in color. The term "stack-burn" often is applied to this form of injury by rice growers and millers. "Stain" and "flecking" are other terms used.

The decay of rice seed in the soil and the staining and decay of the commercial product make investigations for determining causes and

[1] Wolk, P. C. van der. Protoascus colorans, a new genus and a new species of the Protoascineæ group, the source of "yellow grains" in rice. *In* Mycol. Centralbl., v. 3, no. 4, p. 153–157, 1 col. pl. 1913.

means of prevention extremely desirable. Some work was started by the Office of Cereal Investigations in the spring of 1919 at Crowley, La., in cooperation with the Louisiana Agricultural Experiment Station. These investigations were continued for two years, but were given only minor attention because a number of other diseases were considered more important at the time. However, some results were obtained which are thought to be of value, especially to those who may undertake further investigation of these problems.

The investigations reported herein were undertaken with the hope of clearing up the cause of the leaf-spot and seedling blight and, if possible, to find some means of prevention. During the short period of these studies the writer's attention was drawn to what appears to be the more important phase of this problem, namely, the staining and decay of rice in shock and storage. The nature of the marketable product is such that a very small percentage of stain or decay is reflected very decidedly in the grade. As previously mentioned, Van der Wolk states that 0.3 per cent is sufficient to cause rejection in the European markets.

FACTORS FAVORING THE DEVELOPMENT OF THIS DISEASE.

If the harvest season is wet and the rice remains in the fields, especially in shock where it does not dry out, stack-burn is likely to develop. Such rice generally is found of poor quality when milled. This staining of the grain is masked to a certain extent by polishing, but this is only temporary, as the polish gradually disappears and the decay continues to develop. If the rice is stored in a damp place after milling or if not thoroughly dry before storing, it is subject to this type of injury. Repolishing is resorted to at times by millers to mask the discolorations in the rice. This would be more effective if the stains were superficial and were not subject to further development. Rice with slight infections of this type when shipped in damp, warm cars or bottoms for considerable distances, or when allowed to stand for many days under such conditions, may reach its destination with sufficient injury to cause it to be rejected. If not rejected, it would be given an inferior grade on the market. Small flecks, which might escape the observation of the most critical observer, are capable of spreading and destroying the entire kernel when the grain is placed in a warm, damp place. The exact degree of moisture required to cause these stains to develop is not known, but the humid atmosphere of the southern rice sections is known to favor this trouble. High temperatures, which are also common in the South, favor the development of these stains. If the rice is stacked damp or is allowed to go through a sweat after stacking it is likely to become stained if the temperatures remain sufficiently high for a few days.

CAUSES OF INJURY.

The seed-injury phase and symptoms of the trouble were touched upon previously by Godfrey,[2] who reported the occurrence of a leaf-spot on rice in Louisiana in 1916. He said the disease looked very much like the black rust of cereals. Again in 1920, after the writer's investigations were begun, Godfrey[3] reported the occurrence of a seedling disease of rice and states that the cause was found by Dr. H. B. Humphrey to be due to an organism belonging to the *Mycelia sterilia* group of fungi. The fungus was characterized by very small black sclerotia and a white sterile mycelium. He further states that the fungus closely resembles the Sclerotium found associated with the leaf disease reported in 1916. Godfrey found the fungus constantly associated with rice seed grown in Louisiana, particularly the Blue Rose and Honduras varieties. California seed, however, was found to be free from the fungus.

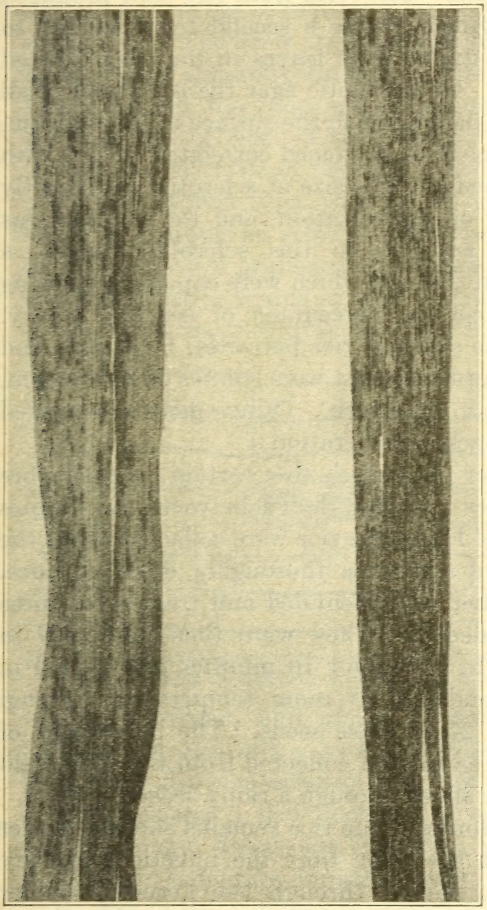


FIG. 1.—Black leaf-spot of rice caused by the sclerotial fungus which produces seed injury and seedling blight.

The cause of these troubles was found to be much more complicated than the notes by Godfrey would seem to indicate. Van der Wolk found the fungus *Protoascus colorans* to be responsible for "yellow grains."[4] The color appeared after the rice had been in heaps for considerable lengths of time. The writer found the small sclerotial fungus of the *Mycelia sterilia* group mentioned by Godfrey[3] to be constantly associated with the leaf-spot (Fig. 1) mentioned by

[2] Godfrey, G. H. Preliminary notes on heretofore unreported leaf disease of rice. (Abstract.) *In* Phytopathology, v. 6, no. 1, p. 97. 1916.

[3] Godfrey, G. H. A seed-borne Sclerotium and its relation to a rice-seedling disease. *In* Phytopathology, v. 10, no. 6, p. 342–343. 1920.

[4] Wolk, P. C. van der. Op. cit.

him and with rice seed grown in the Southern States (Pl. I). However, at least three strains of this fungus were found, basing the difference on size of the sclerotia and the color of pigment produced in certain types of media.

One strain isolated from rice leaves was almost free from pigment in culture; another strain from leaves produced a small quantity of pinkish pigment on most media. One form from rice seed produced an abundance of pinkish pigment, while a second form seemed to be identical with one of the strains from leaves in that it produced a small quantity of pigment. On synthetic agar the mycelium of all forms developed for the most part beneath the surface of the medium, and the size and number of sclerotia produced correlated closely with the pigmentation on other media. The size of sclerotia was directly proportional to the quantity of pigmentation, and the number was inversely proportional. In addition to this sclerotial fungus, a number of fungi were found in the seed which were capable of causing staining and decay and a certain percentage of seedling injury. Among these were some of the common rice parasites, Piricularia and Helminthosporium. Other forms present were species of Epicoccum, Penicillium, Aspergillus, and Eusarium. Other genera occurred rarely and were not given much consideration.

These fungi, no doubt, enter the kernels to a certain extent before they are mature. The spores probably lodge between the glumes at flowering time. Panicles of Honduras rice were collected about the time they were fully mature and were thoroughly dried at once. Seeds from these, 62 in number, were dehulled and treated for three minutes with 70 per cent alcohol. They were then immersed in mercuric-chlorid solution (1 to 1,000) for 10 minutes and placed in tubes of sterile agar and incubated at room temperature. Fungi were found to be present in 27 of these seeds. The percentage of infection was hardly as high as in seed collected from the same field after the rice had stood in the shock through a rainy season.

The infection spreads, no doubt, if the rice remains shocked under warm, moist conditions. At the same time the infections already present have a tendency to spread through the kernels. Under such conditions sclerotia of the sclerotial type of organism are formed in the glumes and on the surface of the kernels. Such infected kernels often are shriveled and very brittle. When conditions are very humid this fungus causes the kernels to become much enlarged and very irregular in shape, with sclerotia resembling those produced by ergot in grain. These distorted kernels are almost black in color (Pls. II and III), and the tissues are filled with a sclerotial development. Where the other fungi mentioned are present the kernels have the brown flecks, and the decayed tissue is a uniform brownish

color. Dark bands of sclerotial tissue may be seen on kernels infected with the sclerotial fungus.

The climatic conditions in the rice-growing sections of the South favor the development of these troubles. The humid atmosphere and high temperatures are conducive to a vigorous growth of the various fungi concerned, and the humidity is unfavorable for the drying and curing of rice, thus leaving it exposed to attacks by these organisms. The drier atmosphere and the rather low night temperatures which generally prevail in the rice-growing sections of California during the crop season probably are not favorable to the development of most of these fungi.

There is one type of flecking known as "blast" in California. In 1919 seed of this type collected at various points in the State was found to contain an Alternaria in the diseased spots. Brown spots or totally brown glumes characterize this trouble. The kernels are also yellowish to brown in color beneath the diseased glumes. Some of the spots have lighter colored centers. The fungus might have been growing as a saprophyte in these dead areas, but the failure to find it associated as constantly with healthy kernels seems to indicate that to some extent it was responsible for the injury. When these kernels were dehulled, surface sterilized, and placed in damp sterile chambers the spots increased in size, and a growth of Alternaria appeared on the surface. Alternaria also was isolated from rice seed grown in Louisiana.

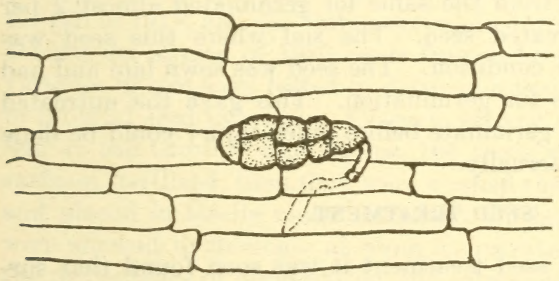


FIG. 2.—Camera-lucida sketch of the germ tube from an Alternaria spore penetrating the epidermis of the leaf of a rice seedling.

In Louisiana a leaf spotting of seedlings was noticed as being quite common before the irrigation water was applied. The spots were small, indefinite in outline, and light brown in color. An Alternaria was found constantly associated with those spots. By examining carefully under the microscope these young leaves, cleared by soaking in equal parts of 95 per cent alcohol and glacial acetic acid, an Alternaria spore could be seen in the center of almost every spot, and often the germ tube could be seen extending from the spore and passing through the epidermis into the leaf tissues (Fig. 2). An Alternaria leaf-spot of black Italian rice and of C. I. No. 1564 was found at Bellflower station, near Los Angeles, Calif., but it was not determined whether or not this was identical with the form producing the "blast" of rice kernels in the Sacramento Valley or with the

Alternaria producing the seedling leaf-spot and seed injury in Louisiana.

Rice infected with the various forms of fungi which produce flecking and decay germinates very poorly as compared with uninfected seed. The germ often is killed by the invading parasite (Pls. II and III, *A*). Wet soils, which tend to check the normal germination and development of seedlings, seem to favor the growth of the fungus, and the seed may be destroyed and a poor stand result. Seed treatment before sowing would not eliminate injury done by these fungi previously, but might prevent further injury and prevent the spread of these organisms. Seed of Honduras rice obtained from California, where seed-infesting fungi are rare, and sown in Louisiana germinated better than seed of the same variety grown in Louisiana (Tables 2 and 4). The difference in the percentages of germination is an indication, perhaps, of the extent of damage commonly caused to seed rice in Louisiana. Of course, if the number of these fungi which may be carried in the soil was considered, the damage might be greater. Treated seed from the same lot germinated almost 2 per cent better than untreated seed. The soil where this seed was sown was in very good condition. The seed was sown late and had almost ideal conditions for germination. This gave the untreated seed an opportunity to germinate before much injury could be done by the fungi carried internally.

SEED TREATMENT.

Upon undertaking a seed treatment it was soon found that surface sterilization was of no value. Seeds were dehulled and treated for 3 minutes in 50 to 70 per cent alcohol and then soaked for 10 minutes in bichlorid of mercury solution (1 to 1,000) with no effect (Pls. IV, *A;* V; and VI, *A*). Godfrey[5] treated dehulled seed by the Jensen modified hot-water method and found that the sclerotial form was killed. Dehulling the seed, of course, would not be at all practicable, so an attempt was made to find out to what extent, if at all, hot water could be used for treating rice in the hull. It was found that practically all the fungi in the seed could be killed without injury to the seed germ if care was exercised in carrying out the treatment.

Rice, which has close-fitting, tough glumes, requires a much longer presoaking in tepid water and a more extended soaking in hot water than the other small grains. The best method found was to presoak the seed overnight in water which was somewhat warm to begin with, and hence more effective than cold water, and then, subsequently, to treat the seed with hot water. Before beginning

[5] Godfrey, G. H. Op. cit. 1920.

the hot-water treatments, tests were made to determine the percentages of rice carrying these fungi internally. The kernels were carefully dehulled, treated 3 to 5 minutes in 50 to 70 per cent alcohol, then soaked for 10 minutes in a 1 to 1,000 solution of mercuric chlorid, and placed in tubes of sterile agar (Pl. IV) or on sterile agar or blotter paper in Petri dishes (Pl. VI) after washing thoroughly in sterile water. The results of these tests are given in Table 1.

TABLE 1.—*Internal infection of rice seeds after surface sterilization in crop varieties grown at Crowley, La., in 1919.*

Variety.	Seeds treated.	Seeds infected.		Variety.	Seeds treated.	Seeds infected.	
		Number.	Per cent.			Number.	Per cent.
Honduras	261	197	75.48	Carolina White	48	46	95.84
Wataribune	60	42	70.00	Shinriki	48	32	66.66

The rice for the tests referred to in Table 1 was taken from the stock seed supply at the Rice Experiment Station, Crowley, La., in the fall of 1919, and the tests were made during the following winter. During the spring of 1920 seeds from a larger number of the best varieties of rice were tested to determine their relative susceptibility to internal seed infection. Of each variety obtained from the seed lots at the Crowley rice station 100 seeds were dehulled and their surfaces sterilized; then they were washed thoroughly in sterile water and placed in sterile moist chambers on damp filter paper. They were allowed to incubate at room temperature for seven days, and the percentages of germination and infection were then taken. No attempts were made to identify the fungi from each kernel. The results of these tests are given in Table 2.

TABLE 2.—*Germination and internal infection of rice seeds grown at Crowley, La., in 1919.*

Variety.	Germination.	Infection.		Variety.	Germination.	Infection.	
	Per cent.	Per cent.	Degree.		Per cent.	Per cent.	Degree.
Delitus (large shocks)	86	50	Slight.	Tokalon	84	38	Slight.
Delitus (small shocks)	64	79	Heavy.	Evangeline	50	95	Heavy.
Blue Rose	92	41	Slight.	Acadia	81	65	Slight.
Vintula	99	35	Do.	Early Prolific	66	49	
Honduras	53	83	Heavy.	Honduras (California)	99	9	Do.
Wataribune	92	25	Slight.	Wataribune (California)	98	7	Do.
Carolina Gold	83	91	Heavy.	Early Prolific (Louisiana)	69	70	Heavy.
Shinriki	63	92	Do.	Honduras (California)	99	25	Slight.
Fortuna	95	38	Slight.	Wataribune (California)	98	8	Do.

In Table 2 the Delitus variety was obtained from both large and small shocks. The harvest season was a very wet one, so the grain in the small shock was injured much more than that in the large or normal shock. The terms "slight" and "heavy," referring to the

degree of infection, represent the rate at which the seedlings were killed or injured by these fungi after the seed germinated. Honduras and Wataribune seed from California, as shown, germinated much better than Louisiana seed of these varieties and were comparatively free from fungi, especially of the types which cause injury.

Honduras rice was selected for the hot-water treatments, as it is a good commercial rice and one of the most susceptible to attack by these internal fungi. Table 3 shows the results of treatments on January 24 and February 2, 1920. The seed was sown in sterile agar plates, 20 seeds per plate.

TABLE 3.—*Effect of hot-water treatment on the germination of Honduras rice seeds and on fungi carried within the seeds.*

Date and number of experiment.	Presoaked in tepid water.	Soaked in hot water.		Germination.	Infection.	Date and number of experiment	Presoaked in tepid water.	Soaked in hot water.		Germination.	Infection.
Jan. 24, 1920: [1]	*Hours.*	*°C.*	*Min.*	*P. ct.*	*P. ct.*	Jan. 24, 1920: [1]	*Hours.*	*°C.*	*Min.*	*P. ct.*	*P. ct.*
No. 1	16½	55	10	83	3	No. 18	([2])	57	15	96	52
No. 2	16½	55	15	78	1	No. 19	([2])	58	15	98	40
No. 3	16½	54	15	87	1	No. 20	([2])	([3])	([3])	96	62
No. 4	16½	56	10	59	13	No. 21	16½	([3])	([3])	92	45
No. 5	16½	56	15	47	3	No. 22	4	([3])	([3])	94	61
No. 6	16½	57	10	43	5	No. 23	2	([3])	([3])	94	67
No. 7	16½	58	10	2	1	Feb. 2, 1920: [4]					
No. 8	4	55	15	93	17	No. 24	16⅔	53	20	90	2
No. 9	4	56	10	91	24	No. 25	16⅔	53	15	91	4
No. 10	4	56	15	85	19	No. 26	16⅔	53	10	86	13
No. 11	4	57	10	66	5	No. 27	16⅔	54	20	76	3
No. 12	4	57	15	75	4	No. 28	16⅔	54	15	93	1
No. 13	4	58	10	72	3	No. 29	16⅔	54	10	81	3
No. 14	4	58	15	74	0	No. 30	16⅔	55	15	71	3
No. 15	2	56	15	89	20	No. 31	16⅔	55	10	91	2
No. 16	2	57	15	78	14	No. 32	16⅔	([3])	([3])	97	52
No. 17	2	58	15	81	15	No. 33	([2])	([3])	([3])	96	47

[1] Soaked at 37° C.; germination in 10 days; infection in 13 days. [2] Not presoaked. [3] Not treated.
[4] Soaking started at 35° C., and the water was then allowed to come to room temperature; germination and infection in 8 days.

Table 3 shows that the long presoaking period is more desirable. In each case the 15-minute treatments at 54° C. gave the best results, in both seed germination and fungus control.

Further tests were made by soaking seed of Honduras rice from Louisiana at 54° C. for 15 minutes, after presoaking 16 hours and 50 minutes in tepid water, and then sowing it along with untreated seed and seed of the same variety which was grown in California. Soil from a field where rice had never been grown was placed in large cans (16 by 16 inches and 15 inches deep), and the 50 and 100 seeds were sown in each can. The soil was in excellent condition, so the seed germinated readily, and none of the seedlings showed signs of blight. Table 4 gives the results of the germination of this seed.

DISEASED AND HEALTHY RICE KERNELS.

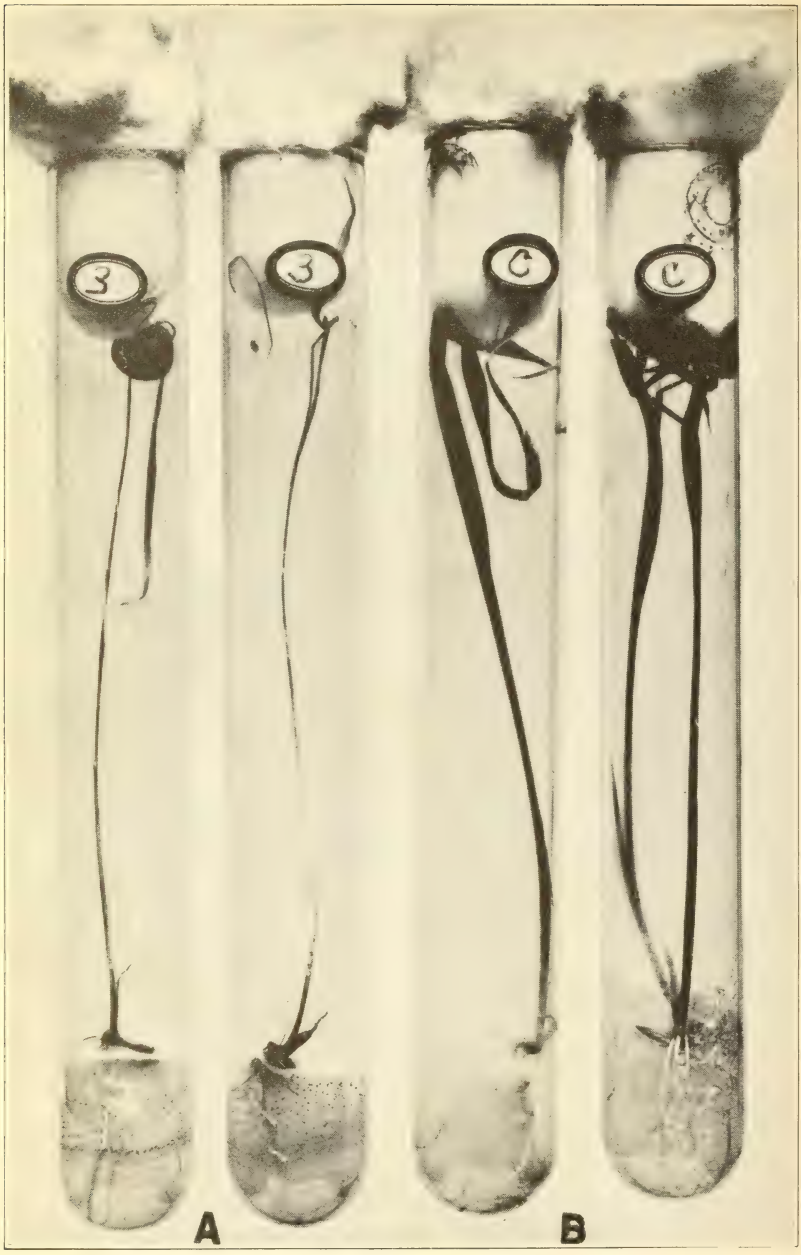
A, Kernels of Honduras rice, enlarged, showing sclerotia on the surface. These kernels were thoroughly invaded by this sclerotial form. *B*, Healthy kernels of the same variety.

DISEASED AND HEALTHY RICE SEEDLINGS AND KERNELS.

At the left is one healthy seedling of Honduras rice. The remainder of the seedlings are infected with fungi, principally the sclerotial form. Note the dark, irregular kernels, which are filled with sclerotial tissue.

INDIVIDUAL RICE KERNELS, SHOWING GERMINATION AFTER SURFACE STERILIZATION AND HOT-WATER TREATMENT.

A, Two kernels of Honduras rice infected with the sclerotial fungus, from a lot which was surface sterilized. *B*, Kernel treated with hot water and sown at the same time as those shown in *A*.

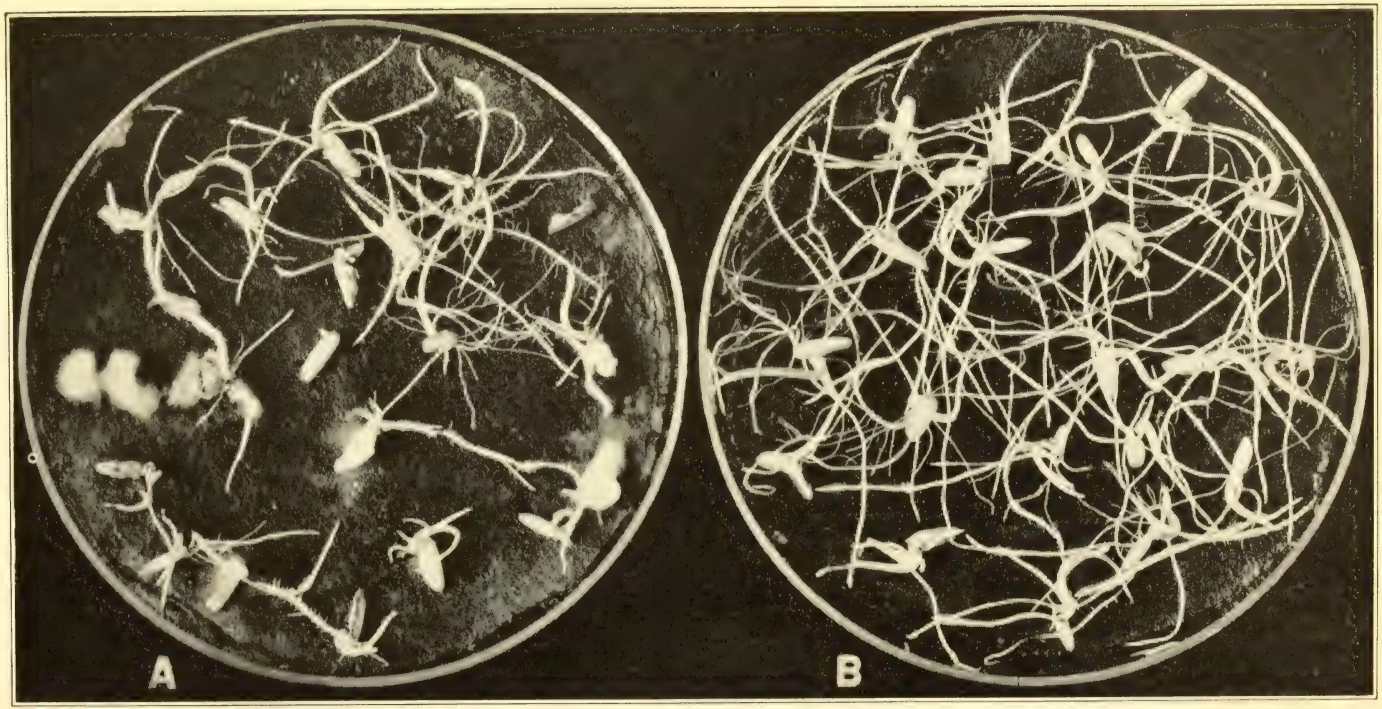


DISEASED AND HEALTHY RICE SEEDLINGS IN CULTURES.

A, Two rice seedlings killed by the sclerotial fungus which infected the seeds. Note the sclerotia in the medium, synthetic agar. *B* Two healthy rice seedlings from clean seed grown in the same medium.

SCLEROTIA ON ROOTS OF RICE SEEDLING.

Test-tube culture, enlarged, showing the sclerotia of the sclerotial fungus developing on the roots of the dead rice seedling. The fungus was carried inside the seed.



GERMINATION OF RICE SEEDS AFTER SURFACE STERILIZATION AND HOT-WATER TREATMENT.

A, Seeds of Honduras rice surface sterilized and sown in sterile Petri dishes on blotter paper. The fungi which developed were carried in the seed. *B*, Uninfected seedlings grown from seed from the same lot as those shown in *A* but soaked 16 hours and treated 15 minutes in water at 51° C.

TABLE 4.—*Germination of Honduras rice seeds from Louisiana treated in water at 54° C. for 15 minutes, after presoaking 16 hours and 50 minutes, as compared with untreated Louisiana and California seeds of the same variety.*

Date sown and number of experiment.	Louisiana seeds.				California seeds.	
	Treated.		Untreated.		Untreated.	
	Number.	Germination.	Number.	Germination.	Number.	Germination.
May 3, 1920:		*Per cent.*		*Per cent.*		*Per cent.*
No. 1			250	52.40	250	55.20
No. 2			250	46.00	250	70.80
No. 3			250	68.40	250	78.40
No. 4			250	64.80	250	64.40
No. 5			250	65.60	250	75.20
No. 6			250	70.80	250	86.40
No. 7			250	72.40	250	84.40
No. 8			250	62.00	250	78.80
May 21, 1921:						
No. 13	400	70.50	200	64.00	400	72.75
No. 14	400	75.00	200	85.50	400	74.00
No. 15	400	65.50	200	65.00	400	63.50
No. 16	500	53.60	500	63.00		
No. 17	500	70.60	500	65.60		
No. 18					1,000	66.10
No. 19	500	63.50			500	73.80
No. 20					1,000	67.00
Total	2,700		3,600		5,700	
Average		65.96		64.80		70.61

Nishikado and Miyake [6] conducted extensive hot-water treatment experiments on the Minaribo variety of rice in Japan. Unfortunately, the results of this work were not available to the writer until after his work on rice was discontinued. These Japanese investigators found that the temperature of the hot water which could be used effectively on rice depended to a large extent on the length of the presoak. They reported no seed injury by treating unsoaked rice for 15 minutes at 55° C. The writer found no injury to Honduras rice when it was treated at 58° C. for 15 minutes without presoak (Table 3).

The Japanese writers mentioned no injury to seed presoaked for different periods up to 16 hours and treated for 5 and 10 minutes in water at 54° C., but a 15-minute soak in hot water caused a slight decrease in germination. Where they presoaked the seed 24 and 48 hours, respectively, a 10-minute soak in hot water at 54° C. caused some injury. Their results also show that seed treated with hot water germinated better than their untreated controls. The writer found that a soaking in water at 54° C. for 15 minutes after presoaking for 16 hours killed the internal fungi and came nearest to giving as good germination as the untreated controls. In practically every case, however, the germination was slightly lower than that of the untreated

[6] Nishikado, Yoshikazu, and Miyake, Giichi. Momidane no shôdoku narabini ine-gomahagarebyô no yobôhô. [Disinfection of seed rice in relation to the prevention of Helminthosporium rice blight.] 1. Momidane no ontô shinsekihô. [Hot-water treatment of seed rice.] *In* Byôchû-gai Zasshi (Jour Plant Protection), v. 5, no. 9, p. 693–712 (1–20). 1918.

controls. The sclerotial fungus seemed to be hardest to kill in Honduras rice.

The Japanese writers were treating for the control of Helminthosporium. They recommended presoaking overnight and soaking with hot water at 54° C. for 5 minutes or at 52° for 10 minutes. They found that the hot-water treatment did not control Piricularia. This was no doubt due to other sources of infection in the field. The hot-water treatment did not prevent the black sclerotial leaf-spot in Louisiana. Treated seed, untreated seed, and clean Honduras seed from California produced plants which were equally diseased. The fungus doubtless lives over in the soil and on old rice straw and infects the rice plants the following season. There are strong indications that this fungus produces conidia which cause the leaf infection. However, this has not been proved definitely. It seems hardly possible that all of the abundant infection which occurs could be caused by sclerotia.

CONCLUSIONS.

The results given in Tables 3 and 4 show that the hot-water treatment will kill the fungi carried within the rice seed and not injure the viability of the seed if the proper methods are used. It also indicates that these fungi have a tendency to lower the vitality of the seed, as is shown by the better germination of seed grown in California, which were relatively free internally from fungi. Hot water does not eliminate this difference but does prevent further development of the fungi in the seed, provided the seed is sown in clean soil where there is no subsequent infection.

Until more information is obtained regarding these fungi and their life histories it would be inadvisable to recommend seed treatment which would kill only the fungi in the seed. Where infested seed is to be sown on virgin soil or soil where rice has not been grown previously, it may be advisable to treat the seed to prevent introducing these fungi into the new lands. Seed from California would be good for this purpose without treating were it not for the numerous weed seeds that might be introduced in this way. The matter of soil infestation by the various fungi concerned has not been investigated sufficiently to show what part it might play in seed decay and seedling blight.

From a commercial standpoint, some form of treatment should be profitable where rice is to be stored or shipped under warm, moist conditions. The hot-water treatment would be too tedious and expensive for treating the commercial product. It is hoped that through future investigations some practicable form of treatment will be discovered. A process of drying under temperatures that would kill the fungi and not injure the texture of the grain might be possible. Van der Wolk [7] recommended the thorough drying of rice and dry

[7] Wolk, P. C. van der. Op. cit.

storage to prevent "yellow grains." Unless the fungi are killed in the process, drying will not serve the desired purpose, as the fungus causing the stains will start developing again if the grain is placed in a warm, damp place.

The most promising solution for these troubles seems to be in the eventual development of resistant strains and varieties. Table 2 shows that some varieties are much more susceptible than others when grown under the same conditions. The varieties which showed the heaviest attacks in these tests are as a rule less hardy and poorer yielders in the field. These seed-infesting fungi evidently have played their part in the varietal selection work where hardy varieties have resulted. With a thorough knowledge of these troubles the problem of breeding hardy strains of rice would be somewhat simplified.

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